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Use of picric acid and iodine as electron acceptors for spectrophotometric determination of lansoprazole through a charge-transfer complexation reaction

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Abstract

This article describes the development of two simple and selective spectrophotometric methods for the determination of lansoprazole (LAN), an irreversible proton pump inhibitor, in both pure drug and capsule formulations. The methods are based on the formation of charge-transfer (CT) complexes between LAN an electron donor and either picric acid or iodine as an electron acceptor. The intensely coloured products formed were quantified based on the absorption bands at 410 nm for picric acid (method A) and 360 nm for iodine (method B). The accuracy and precision of the methods were evaluated on intra-day and inter-day bases. Beer's law is obeyed in the concentration ranges of 2–32 and 0.8–12.0 μg/ml LAN for method A and method B, respectively. The molar absorptivity values, limits of detection (LOD) and limits of quantification (LOQ) have also been reported. The reaction stoichiometry for both methods was evaluated by Job's method of continuous variation and was found to be 1:1 (LAN: picric acid and LAN: iodine). The proposed methods were successfully applied to the determination of LAN in capsules with good accuracy and precision and without a detectable interference from common excipients. A statistical comparison of the methods revealed that there is no significant difference between the official method and the proposed methods.

Keywords: Lansoprazole; Spectrophotometry; Picric acid; Iodine; CT complex; Pharmaceutical analysis

1. Introduction

Lansoprazole (LAN), which is chemically known as 2-[[[3-methyl-4-(2,2,2 trifluoroethoxy) pyridine-2-yl]methyl]sulfinyl]-1*H*-benzimidazole, is widely used as an anti-ulcer drug (proton pump inhibitor) through inhibition of H^+, K^+-ATP-ase in gastric parietal cells [1]. The drug assay is listed in the monograph of the United States Pharmacopoeia (USP) [2] and the British Pharmacopoeia (BP) [3]. The USP describes a high-performance liquid chromatographic method and the BP recommends

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a potentiometric titration of LAN with NaOH in a 4:1 ethanol:water mixture.

Several methods have been reported for the determination of LAN in pharmaceutical formulations, including high-performance liquid chromatography (HPLC) [4–17], ultra-performance liquid chromatography (UPLC) [18–21], high-performance thin-layer chromatography (HPTLC) [22,23], liquid chromatography/tandem mass spectrometry (LC–MS) [24], capillary electrophoresis [25,26], polarography [27–29], voltammetry [30], UV spectrophotometry [31–38], flow-injection analysis (FIA) [12,39], kinetic spectrophotometry [40,41], spectrofluorimetry [42,43] and fluorimetry [33]. Although those methods are sensitive, some of them are time-consuming, complicated, and require expensive instrumentation. In particular, chromatographic methods necessitate judicious control of the pH of the medium. Therefore, visible spectrophotometry remains the technique of choice because it is sensitive, economical, rapid and easily manageable.

A number of colour formation reactions utilizing different reagents have been employed for the visible spectrophotometric determination of LAN in pharmaceuticals [4,12,40,44–56]. The reported methods are based on complexation and oxidative coupling [44], formation of a charge-transfer complex [4,45], redox followed by complexation or colour bleaching [46–49], bromination [50], ion-pair complexation reaction [51–55] and coupling with diazotized p-nitroaniline [56]. However, most of the reported visible spectrophotometric methods suffer from one or more disadvantages, such as poor sensitivity [4,44,45], a narrow range of determination [47–50], use of a heating step [44,48], and use of an extraction step [52–55], as shown in Table 1.

The present work describes two rapid and simple visible spectrophotometric methods for the determination of LAN by exploiting its basic nature and electron-donating property. This determination is based on a charge-transfer complexation of LAN with either picric acid as a π-acceptor or iodine as a σ-acceptor. Iodine has been used for the spectrophotometric determination of LAN based on a charge-transfer complexation reaction in a chloroform medium [45]. In the present study, the same reaction in a dichloromethane medium was found to be very rapid and far more sensitive with a wide linear dynamic range. The proposed methods utilizing picric acid and iodine as reagents in dichloromethane were successfully applied to the determination of LAN, in either its pure form or in capsules, with good accuracy and precision.

2. Experimental

2.1. Instrument

A Systronics model 106 digital spectrophotometer provided with 1-cm matched quartz cells was used for all absorbance measurements.

2.2. Materials

Pharmaceutical-grade LAN with a certified purity of 99.80% was obtained from Cipla Ltd., Bangalore, India. The following pharmaceutical preparations were purchased from commercial sources and subjected to analysis: Lan-15 (15 mg LAN per capsule) and Lan-30 (30 mg LAN per capsule) from Intas Pharmaceuticals, Dehradun, India; Lanzol-15 and Lanzol-30 from Cipla Ltd., Sikkim, India.

2.3. Reagents and chemicals

All reagents and solvents used were of analytical-reagent grade. Picric acid (0.4%, w/v) (S.D. Fine Chem. Ltd., Mumbai, India) and iodine (0.1%, w/v) (Loba Chemie, Mumbai, India) solutions were prepared in dichloromethane (DCM) (Merck, Mumbai, India) and kept in the dark when not in use.

2.4. Stock solution of LAN

Using a 100-ml calibrated flask, a 100-ml stock solution (100 μg/ml LAN) was prepared by dissolving an accurately weighed 10 mg aliquot of the pure drug in DCM. This solution was further diluted with DCM to obtain working concentrations of 40.0 and 20.0 μg/ml LAN for use as standards in methods A and B, respectively.

2.5. Sample preparation

2.5.1. Capsules

The contents of 20 capsules were combined, mixed, weighed accurately and ground to a powder. A portion of the powder equivalent to 5 mg of LAN was accurately weighed and transferred into a 50-ml calibrated flask. Then, 30 ml of DCM was added to the flask, and the container was shaken thoroughly for 15–20 min to extract the drug into the liquid phase. Finally, the solution was diluted to the mark with the same solvent, mixed well and filtered using Whatman No. 42 filter paper. An aliquot of the filtrate (100 μg/ml LAN) was further diluted with DCM to obtain working concentrations of

Table 1
Comparison of the performance characteristic of the existing visible spectrophotometric methods with the proposed methods for the determination of LAN in pharmaceuticals.

Sl. No.	Reagent/s used	λ_{max}, nm	Linear range (μg/ml), ε ($L\,mol^{-1}\,cm^{-1}$)	Remarks	Ref.
1	Chloranilic acid in acetonitrile	520	5–80 ($\varepsilon = 3.45 \times 10^3$)	Less sensitive	[4]
2	NBS-chloranilic acid using FIA system	530	5–150	Less sensitive	[12]
3	(a) $KMnO_4$–NaOH (b) $KMnO_4$–NaOH kinetic studies	610 530	5–150 5–70	Less sensitive, rate is critically dependent on experimental variables	[40]
4	(a) Acetyl chloride with $CuSO_4$ (b) MBTH with ceric ammonium sulphate	478.5 491.2	100–600 100–500	Less sensitive and requires heating at 100 °C for 5 min	[44]
5	(a) DDQ in acetonitrile (b) Iodine in chloroform (c) Ternary complex formation with Eosin and copper(II)	457 293 & 359 549	10–90 ($\varepsilon = 4.10 \times 10^3$) 1.48–6.65 ($\varepsilon = 2.72 \times 10^4$ & 5.65×10^4) 3.69–16.61 ($\varepsilon = 1.58 \times 10^4$)	Requires heating at 60 ○C for 20 min for ternary complex formation	[45]
6	Ceric ammonium sulphate and iron(II) with (a) orthophenanthroline; (b) thiocyanate	510 470	2.5–30 ($\varepsilon = 8.1 \times 10^3$) 2.5–25 ($\varepsilon = 1.5 \times 10^4$)	Lengthy procedure and also involve multiple-step reactions	[46]
7	Ceric ammonium sulphate with (a) methyl orange and (b) indigo carmine	520 610	0.5–7 ($\varepsilon = 3.0 \times 10^4$) 0.25–3 ($\varepsilon = 4.4 \times 10^4$)	Sensitive but involve multiple-step reactions, narrow linear range	[47]
8	Copper(II) sulphate with (a) neocuproine and (b) bathocuproine	460 480	0.2–3.6 ($\varepsilon = 5.82 \times 10^4$) 0.2–3.2 ($\varepsilon = 7.17 \times 10^4$)	Sensitive but requires heating on a boiling water bath for 10 min and narrow linear range	[48]
9	Iron(III) with ferricyanide	730	0.2–3.6 ($\varepsilon = 6.78 \times 10^4$)	Sensitive but uses a slow reaction	[49]
10	Bromate-bromide mixture and iron(II) with (a) thiocyanate and (b) orthophenanthroline	470 510	0.5–4 ($\varepsilon = 3.97 \times 10^4$) 0.5–6 ($\varepsilon = 3.07 \times 10^4$)	Involve multiple-step reactions	[50]
11	(a) BCP in DCM (b) BTB in DCM	400 430	0.5–15 ($\varepsilon = 2.10 \times 10^4$) 1.25–20 ($\varepsilon = 1.50 \times 10^4$)	–	[51]
12	BCG	416	1–20	Extraction step is required	[52]
13	(a) Supracen Violet 3B (b) Tropaeolin OOO (c) CAT- Gallocyanine (d) NBS- Celestin Blue	590 500 540 540	5–40 ($\varepsilon = 0.9232 \times 10^4$) 5–25 ($\varepsilon = 1.0857 \times 10^4$) 2.5–12.5 ($\varepsilon = 7.0997 \times 10^4$) 1–6 ($\varepsilon = 2.3265 \times 10^4$)	Extraction step is required in a & b, NBS solution unstable	[53]
14	(a) BTB (b) BPB (c) BCP (d) BCG	419 417 416 414	2.5–25 ($\varepsilon = 18{,}596$) 4–30 ($\varepsilon = 20{,}333$) 4–40 ($\varepsilon = 22{,}630$) 4.5–45 ($\varepsilon = 25{,}622$)	Extraction step is required	[54]
15	(a) Metanil yellow (b) Methyl orange	440 450	20–70 6–16	Extraction step is required	[55]
16	Diazotized p-nitroaniline in alkaline medium-DMF	610	10–35		[56]
17	(a) Picric acid in DCM (b) Iodine in DCM	410 360	2–32 ($\varepsilon = 9.24 \times 10^3$) 0.8–12 ($\varepsilon = 3.64 \times 10^4$)	Sensitive, no heating or extraction step, no pH-adjustment, single step reaction	Present methods

ε, molar absorptivity; NBS, N-bromosuccinimide; FIA, flow injection analysis; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; MBTH, 3-methyl-2-benzothiazolinone hydrazine; BCP, bromocresol purple; DCM, dichloromethane; BTB, bromothylmol blue; BCG, bromocresol green; CAT, chloramine T; BPB, bromophenol blue; DMF, dimethylformamide.

40 and 20 μg/ml LAN for use in methods A and B, respectively.

2.5.2. Placebo

A placebo was composed of talc (20 mg), starch (15 mg), acacia (15 mg), methyl cellulose (20 mg), sodium citrate (15 mg), magnesium stearate (20 mg) and sodium alginate (15 mg); its solutions was prepared as described under "capsules".

2.5.3. Synthetic mixture

To the placebo blank, 5 mg of LAN was added and homogenized and then transferred to a 50-ml calibrated flask. Solutions were prepared as described under "capsules".

2.6. Procedures

2.6.1. Method A (using picric acid)

Different aliquots (0.25, 0.5, 1.0, 2.0, 3.0 and 4.0 ml) of a standard LAN (40 μg/ml) solution in DCM were transferred to a series of 5-ml calibrated flasks using a micro burette. To each flask, 1 ml of 0.4% picric acid solution was added; the mixture was diluted to the desired volume with DCM and mixed well. After 10 min, the absorbance of each solution was measured at 410 nm against a reagent blank.

2.6.2. Method B (using iodine)

Varying aliquots (0.20, 0.5, 1.0, 2.0 and 3.0 ml) of a standard LAN (20 μg/ml) solution were transferred into a series of 5-ml calibrated flasks as described above. To each flask was added 1 ml of 0.1% iodine solution, and the mixture was diluted to the desired volume with DCM and mixed well. The absorbance of each solution was measured at 360 nm against a reagent blank.

In both methods, a standard calibration curve was prepared by plotting the absorbance *versus* concentrations of LAN, and linear equations for the standard curves were calculated by linear regression.

2.6.3. Procedure for stoichiometric ratio

To establish the mole ratio between LAN and the reagents used, Job's method of continuous variations of equimolar solutions was employed. The solutions equivalent to 1.083×10^{-4} and 5.414×10^{-5} M LAN were prepared by dissolving the calculated amounts of LAN in DCM. Further, 1.083×10^{-4} M picric acid and 5.414×10^{-5} M iodine solutions were prepared in DCM. A series of solutions was prepared in which the total volume of LAN and reagent was maintained at 5 ml. The drug and the reagent were mixed in various proportions (0:5, 1:4, 2:3, 3:2, 4:1 and 5:0), and the absorbance of the resultant charge-transfer complex was measured at 410 nm in method A and at 360 nm in method B. The absorbance was then plotted against the mole fraction of the drug, [drug]/([drug] + [reagent]).

3. Results and discussion

Mulliken's theory of charge-transfer complexation reactions has been extensively applied to the spectrophotometric determination of several drugs containing an electron-donating group [57–62]. The charge-transfer complex forming reactions are based on the concept that π- and σ-acceptors react with basic nitrogenous compounds (n-donor) to form charge-transfer complexes or radical anions depending on the polarity of the solvent used. LAN, being a basic nitrogen-containing compound, was used with picric acid (as a π-acceptor) and iodine (as a σ-acceptor) in the proposed methods.

3.1. Reaction of LAN with π-acceptor (picric acid)

The interaction of LAN with picric acid in a DCM medium was found to yield an intense yellow-coloured charge-transfer complex. The absorption spectra of the coloured product and the reagent blank were recorded between 380 and 500 nm and showed that both blank and sample absorb maximally at 380 nm. However, the maximum difference in absorbance between the sample and the blank was observed at 410 nm, as shown clearly in Fig. 1. Hence, all measurements were made at 410 nm against a reagent blank. The interaction between LAN (the n-donor) and picric acid (the π-acceptor) is a

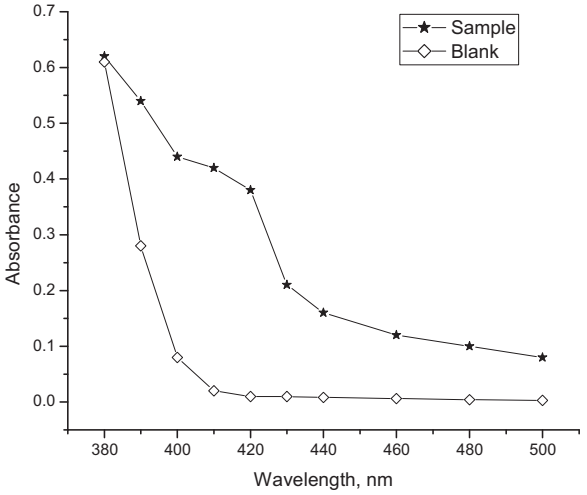

Fig. 1. Absorption spectra for method A (16 μg/ml LAN).

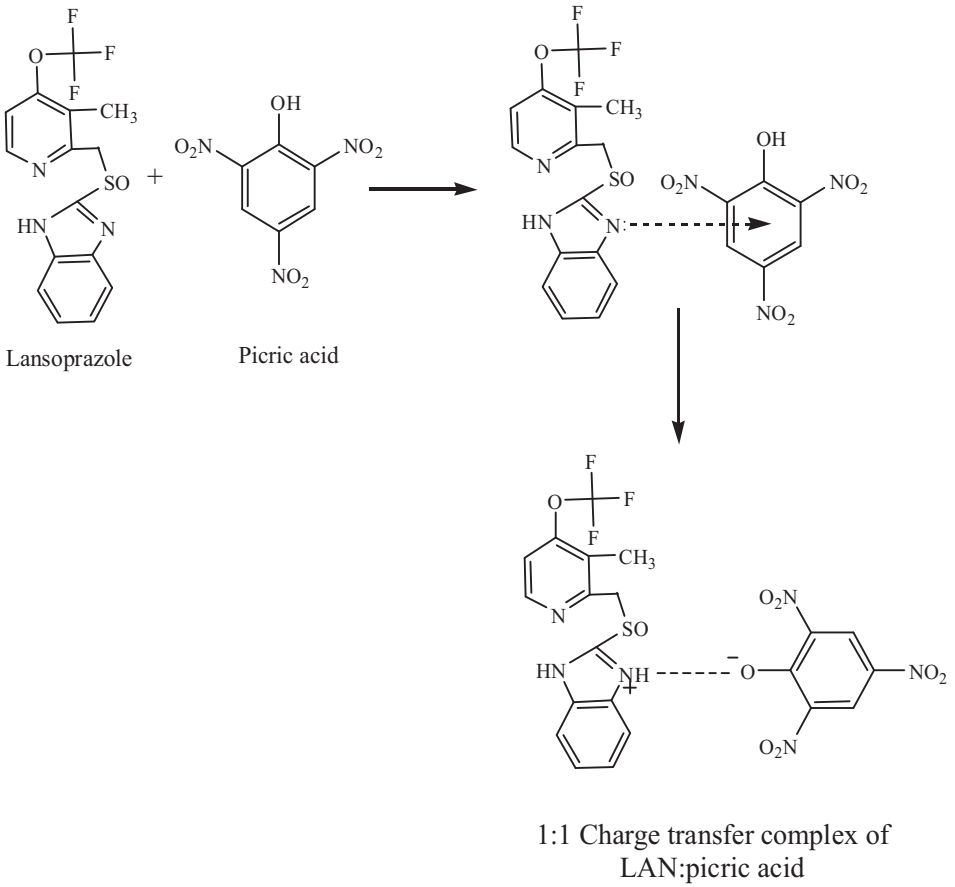

Fig. 2. Possible reaction pathway of a charge-transfer complex between LAN and picric acid.

charge-transfer complexation reaction based on the transfer of a proton (H^+ ion) from the hydroxyl group of picric acid to the nitrogen atom in the benzimidazole ring of LAN. The fact that picric acid produces a colourless solution in DCM, which becomes yellow upon adding LAN, shows that picric acid is not dissociated in the DCM solution. These observations also indicate that picric acid functions as a proton donor in the presence of LAN; the development of a yellow colour is due to the formation of the picrate ion (phenolate ion) [63]. The possible reaction pathway of LAN and picric acid is shown in Fig. 2.

3.2. *Reaction of LAN with σ-acceptor (iodine)*

An increase in the absorbance of the violet colour of iodine in DCM (a blank solution) was observed from 390 nm onwards (Fig. 3) due to the electronic transition of free iodine [64,65]. The addition of LAN resulted in a hypsochromic shift due to the charge transfer complexation. Thus, the new band at 360 nm (Fig. 3) is assigned as a charge-transfer (CT) absorption band. The observed blue shift of the free iodine band upon complexation could be attributed to a perturbation of the iodine molecular orbital (σ*) by a repulsive interaction between LAN

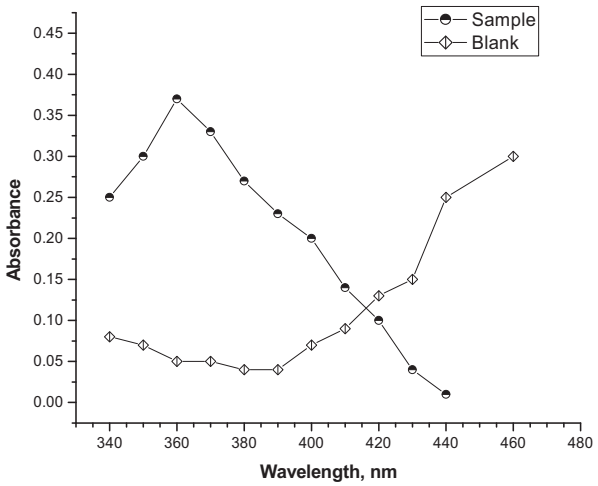

Fig. 3. Absorption spectra for method B (4 μg/ml LAN).

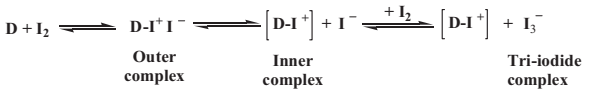

Fig. 4. Reaction pathway for the formation of a tri-iodide complex.

and the iodine molecule in the CT complexes [58,66]. Formation of the tri-iodide complex as a result of the interaction between the n-donor (D) and the σ-acceptor (I_2) has been reported [58,67]. The reaction pathway for this reaction is shown in Fig. 4 and can be summarized by the following steps: (a) formation of the associative outer-sphere CT complex $D{-}I^{+}I^{-}$, (b) transformation to the dissociative inner-sphere complex $[D{-}I^{+}]{+}I^{-}$, and (c) association with another iodine molecule to form the tri-iodide complex $I_3{}^{-}$. The lone pair of electrons on benzimidazole nitrogen atom in LAN emphasized this interaction pathway.

3.3. Optimization of reaction conditions

3.3.1. Effect of solvent

To select a suitable solvent for CT complex formation, the reaction of LAN with picric acid and iodine was monitored in different solvents, namely, DCM, 1,2-dichloroethane (DCE), chloroform, benzene, acetonitrile (ACN), dioxane and methanol. In both methods, the sensitivity of the reaction was found to be very high in ACN; however, the reagent blank was unstable in this medium. DCM demonstrated superiority over other solvents in terms of sensitivity, minimum blank absorbance and stability of both blank and sample (Fig. 5a and b). Hence, DCM was used as the reaction medium in addition to being the solvent for solutions of LAN and the reagents (picric acid and iodine).

3.3.2. Effect of reagent concentration

The effect of reagent concentration on the absorption intensities for the selected wavelengths was ascertained using different volumes of reagents, picric acid (0.4%) in method A and iodine (0.1%) in method B. In method A, the blank absorbance was found to increase with an increase in concentration of picric acid, and 1.0 ml of 0.4% picric acid was found to provide maximum sensitivity with a minimum blank absorbance (Fig. 6). Hence, 1 ml of 0.4% picric acid in a total volume of 5 ml was used throughout the experiment. In method B, both sensitivity and the blank absorbance were found to increase with increasing concentrations of the iodine, as shown in Fig. 6. Considering the minimum blank absorbance and the sensitivity, 1 ml of 0.1% iodine was fixed in a total volume of 5 ml for method B.

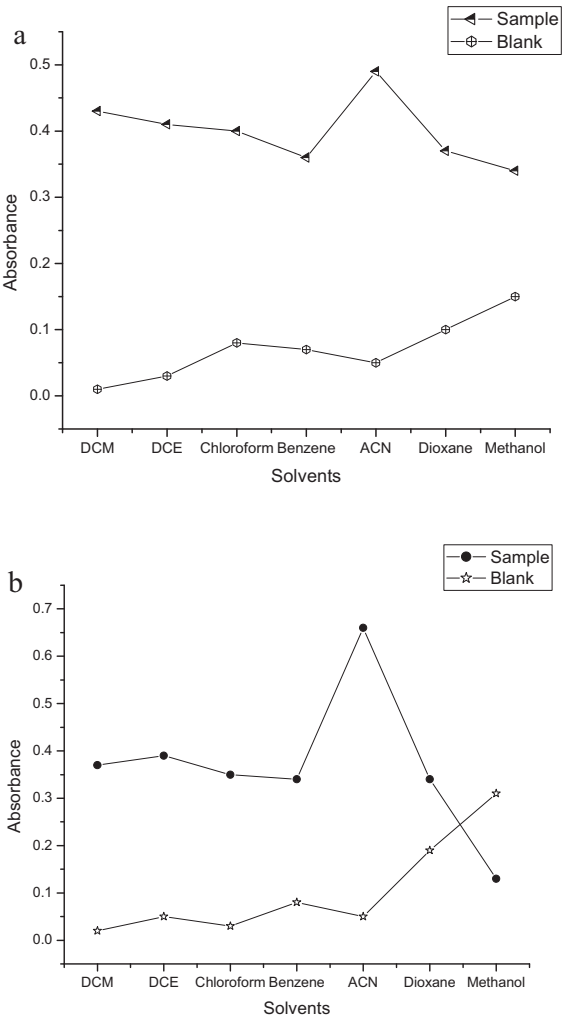

Fig. 5. Effect of solvents on the colour development: (a) method A (16 μg/ml LAN); (b) method B (4 μg/ml LAN).

3.3.3. Effect of reaction time and stability of the reaction product

The optimum reaction time for the development of colour at ambient temperature (25 ± 2 °C) was studied, and it was determined that complete colour development was achieved after 10 min for method A. In method B, the reaction was found to be instantaneous. The formed colour was stable for at least 2 h in method A and 30 min in method B.

3.4. Stoichiometry

The molar ratio of LAN to π- or σ-acceptor (picric acid or iodine, respectively) in the formed complexes was determined by applying Job's method of continuous variations. In both cases, the plot reached a maximum value

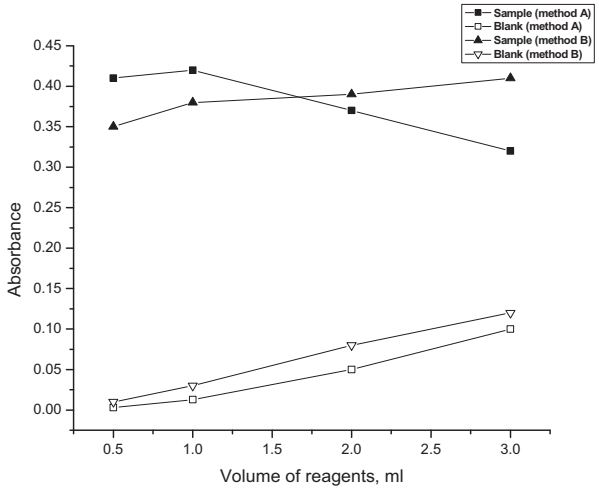

Fig. 6. Effect of reagents: (16 μg/ml LAN and 0.4% picric acid in method A; and 4 μg/ml LAN and 0.1% iodine in method B).

at a mole fraction of 0.5, which indicated the formation of a 1: 1 (LAN: picric acid or iodine) complex, as shown in Fig. 7a and b. The conditional stability constants (K_f) of the charge-transfer complexes were calculated using the data from the continuous variations method in the following equation [68]:

$$K_f = \frac{A/A_m}{(1 - A/A_m)^{n+2} C_M(n)^n}$$

where A and A_m are the observed maximum absorbance and the absorbance value when all of the drug present is associated, respectively; C_M is the molar concentration of the drug at maximum absorbance; and n is the stoichiometry. This resulted in K_f values of 3.19×10^7 and 1.46×10^7 for LAN-picric acid and LAN-iodine complexes, respectively.

4. Method validation

4.1. Analytical data

Under optimum experimental conditions, the absorbance responses were linear in relation to the concentration of LAN over the ranges of 2–32 μg/ml in method A and 0.8–12.0 μg/ml in method B. The calibration graph in each instance is described by the equation:

$$Y = a + bX$$

where Y = absorbance, a = intercept, b = slope and X = concentration in μg/ml; the fit was obtained by the method of least squares. The molar absorptivity, Sandell's sensitivity, correlation coefficient, limit of detection (LOD), limit of quantification (LOQ), standard

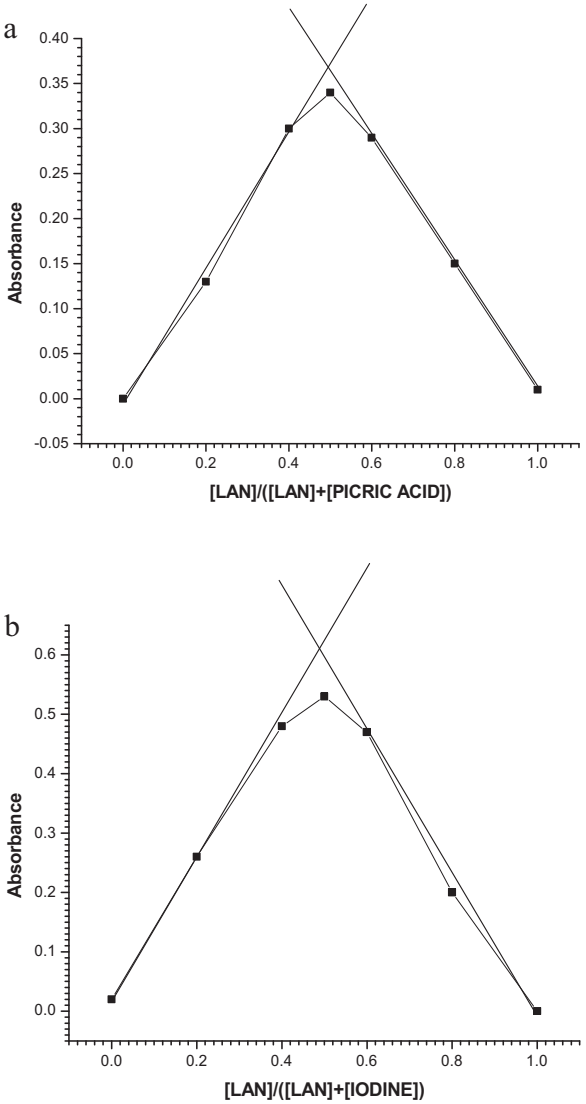

Fig. 7. Job's continuous – variations plots: (a) for method A and (b) for method B.

deviation of intercept (S_a) and standard deviation of slope (S_b) for both methods are calculated as per the current ICH guidelines [69] and are summarized in Table 2.

4.2. Accuracy and precision

To evaluate the accuracy and precision of the proposed methods, solutions containing three different concentrations of LAN were prepared and analyzed in seven replicates during the same day (intra-day) and over five consecutive days (inter-day), and the results are summarized in Table 3. The low values of the relative standard deviation (RSD $\leq$ 1.97% for intra-day) and (RSD $\leq$ 2.71% for inter-day) indicate the high precision

Table 2
Sensitivity and regression parameters.

Parameter	Method A	Method B
λ_{max}, nm	410	360
Linear range, μg/ml	2–32	0.8–12.0
Molar absorptivity (ε), L mol^{-1} cm^{-1}	9.24×10^3	3.64×10^4
Sandell sensitivity[a], μg/cm^2	0.04	0.01
Limit of detection (LOD), μg/ml	0.62	0.08
Limit of quantification (LOQ), μg/ml	1.89	0.23
Regression equation, Y[b]		
Intercept (a)	0.002	0.014
Slope (b)	0.026	0.09
Standard deviation of a (S_a)	0.015	0.126
Standard deviation of b (S_b)	0.0005	0.012
Regression coefficient (r)	0.999	0.999

[a] Limit of determination as the weight in μg per ml of solution, which corresponds to an absorbance of $A = 0.001$ measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm.
[b] $Y = a + bX$, where Y is the absorbance, X is concentration in μg/ml, a is intercept, b is slope.

of the proposed methods. Additionally, the accuracy of the proposed methods was evaluated in terms of relative error (% RE), and from the results shown in Table 3, it is clear that the accuracy is good (RE ≤ 2.81%).

4.3. Selectivity

The selectivity of the proposed methods was evaluated by analysis of a placebo blank solution, and the resulting absorbance readings in both methods were the same as the reagent blank, indicating that no interference from the placebo occurred. Non-interference from the placebo was further confirmed by carrying out a recovery study from the synthetic mixture with percent recoveries of 103.8 ± 2.02 and 97.96 ± 1.96 for method A and method B, respectively. These results confirm the selectivity of the proposed methods in the presence of commonly employed excipients.

4.4. Robustness and ruggedness

Robustness and ruggedness were checked at three different drug levels. The method robustness was evaluated by making small incremental changes in two experimental variables, reagent volume and reaction time. The effect of the changes in the absorbance reading of the resulting complexes in both methods was found to be negligible, thereby confirming the robustness of the proposed methods. To check the ruggedness, analysis was performed by four different analysts and on three different spectrophotometers by the same analyst. The intermediate precision, expressed as percent RSD, is a measure of robustness and ruggedness and was determined to be within the acceptable limits, as shown in Table 4.

4.5. Application to analysis of capsules

The proposed methods were successfully applied to the determination of LAN in commercial capsules. The results obtained by the proposed methods were compared to those of the reference method [3] by applying Student's t-test for accuracy and the F-test for precision at the 95% confidence level. The reference method involved the potentiometric titration in which LAN in a 4:1 ethanol:water mixture was titrated against 0.1 M NaOH. The results in Table 5 show that Student's t- and F-values at the 95% confidence level are less than the theoretical values, which confirmed that there is good agreement between the results obtained using the proposed methods and those obtained using the reference method with respect to accuracy and precision.

4.6. Recovery studies

The accuracy and validity of the proposed methods were further evaluated by performing recovery studies. Pre-analyzed capsule powder was spiked with pure LAN at three concentration levels (50%, 100% and 150%

Table 3
Evaluation of intra-day and inter-day accuracy and precision.

Method	LAN taken, μg/ml	Intra-day accuracy and precision ($n = 7$)			Inter-day accuracy and precision ($n = 5$)		
		LAN found, μg/ml	%RE[a]	%RSD[b]	LAN found, μg/ml	%RE	%RSD
Method A (using picric acid)	16.0	16.35	2.19	1.97	16.45	2.81	2.15
	24.0	24.54	2.25	1.85	24.65	2.71	2.69
	32.0	32.58	1.81	1.79	32.79	2.47	2.21
Method B (using iodine)	4.0	3.94	1.50	1.19	4.10	2.50	1.63
	6.0	6.04	0.67	1.24	6.10	1.67	1.49
	8.0	8.10	1.25	1.11	7.85	1.88	1.73

[a] RE: Relative error.
[b] RSD: Relative standard deviation.

Table 4
Robustness and ruggedness expressed as intermediate precision (%RSD).

Method	LAN taken, μg/ml	Method robustness		Method ruggedness	
		Parameter altered		Inter-analysts' %RSD, ($n=4$)	Inter-instruments' %RSD, ($n=3$)
		Volume of picric acid/iodine[a] ($n=3$)	Reaction time[b] ($n=3$)		
Method A (using picric acid)	16.0	2.17	1.75	1.42	2.26
	24.0	1.93	1.82	1.27	2.41
	32.0	2.05	1.79	1.21	2.31
Method B (using iodine)	4.0	1.33	–	0.87	2.19
	6.0	1.27	–	1.05	2.28
	8.0	1.21	–	0.94	2.17

[a] The volumes of picric acid in method A and the volumes of iodine in method B were 0.8, 1.0 and 1.2 ml.
[b] In method A, the reaction times were 9, 10 and 11 min.

Table 5
Results of analysis of capsules by the reference and proposed methods.

Capsule Brand name	Label claim, mg/capsule	Found[a] (Percent of label claim ± SD) ($n=5$)		
		Reference method	Method A (using picric acid)	Method B (using iodine)
Lan-15	15	100.7 ± 0.84	101.3 ± 1.11 $t=0.97$ $F=1.75$	99.42 ± 0.92 $t=2.30$ $F=1.20$
Lanzol-15	15	100.5 ± 0.89	101.7 ± 1.04 $t=1.96$ $F=1.37$	98.97 ± 0.99 $t=2.57$ $F=1.24$
Lan-30	30	101.2 ± 0.97	102.5 ± 1.13 $t=1.96$ $F=1.36$	101.9 ± 1.08 $t=1.08$ $F=1.24$
Lanzol-30	30	99.87 ± 1.00	98.17 ± 1.07 $t=2.60$ $F=1.14$	99.23± 1.02 $t=1.00$ $F=1.04$

[a] Mean value of five determinations.
The value of t (tabulated) at 95% confidence level and for four degrees of freedom is 2.78.
The value of F (tabulated) at 95% confidence level and for four degrees of freedom is 6.39.

Table 6
Accuracy assessment by recovery experiment.

Method	Capsule studied	LAN in capsule, μg/ml	Pure LAN added, μg/ml	Total found, μg/ml	Pure LAN recovered[a] Percent ± SD
Method A (using picric acid)	Lan-15	8.10	4.0	12.22	103.0 ± 1.21
		8.10	8.0	16.4	103.8 ± 1.42
		8.10	12.0	19.96	98.83 ± 1.37
Method B (using iodine)	Lan-15	3.98	2.0	6.05	103.5 ± 1.14
		3.98	4.0	7.90	98.00 ± 1.26
		3.98	6.0	10.11	102.2 ± 1.08

[a] Mean value of three measurements.

of that in capsule powder), and the total was determined using the proposed methods. In both cases, the added LAN recovery percentage values ranged from 98.00 to 103.8% with a standard deviation of 1.08–1.42 (Table 6), indicating that the recovery was good and that the co-formulated substance did not interfere in the determination of concentration.

5. Conclusions

Two simple and rapid spectrophotometric methods for the determination of LAN in capsules were developed and validated as per the ICH guidelines. These methods are based on well-characterized charge-transfer complexation reactions utilizing picric acid and iodine

as analytical reagents. Compared with most of the existing methods for the determination of LAN, the present methods are very simple and cost effective because they involve simple mixing of the drug and reagent solutions in dichloromethane and measuring the absorbance. Although iodine has been used previously for the determination of LAN in a chloroform medium [45], the proposed method of LAN-iodine in a DCM medium is found to be superior to the reported method with respect to sensitivity, rapidity and linear range. The previously reported method determined LAN over a concentration range of 1.48–6.65 μg/ml with a molar absorptivity value of 2.72×10^4 l/(mol cm) and required 5 min of reaction time. The proposed LAN-iodine method determines LAN in concentrations from 0.8 to 12.0 μg/ml with a molar absorptivity of 3.64×10^4 l/(mol cm), and the reaction was found to be instantaneous. In contrast to many published methods for the determination of LAN, the present methods can be applied at ambient temperature and require neither a complicated extraction procedure nor strict pH control. These merits, coupled with high selectivity and the use of simple, inexpensive instrumentation, suggest that these methods are well suited for use in routine quality control laboratories.

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